ALFRED I. DU PONT INSTITUTE
OF
THE NEMOURS FOUNDATION
P. O. BOX 269
WILMINGTON, DELAWARE 19899
(302) 655-6386

GROVE G. WILEY, M.D.
T. TIMOTHY MYODA, PH.D.
MICROBIOLOGY

PAUL B. HAMILTON, M.D., PH.D.
RICHARD HOLMES, PH.D.
BIOCHEMISTRY

G. DEAN MacEWEN, M.D.
MEDICAL DIRECTOR AND
SURGEON-IN-CHIEF

May 9, 1973

Dr. Frederic W. Nordsiek
Associate Scientific Director
The Council for Tobacco Research -- U.S.A., Inc.
110 East 59th Street
New York, New York 10022

Re: Your Grant Application #907

Dear Dr. Nordsiek:

The program of research titled, "Effect of Smoke Constituents on the Composition of Connective Tissue Proteins Elaborated In Vitro," proposed by Dr. Ines Mandl and associates of Columbia University College of Physicians and Surgeons is very ambitious. The project covers a considerable number of parameters in the fields of Tissue Culture, Protein Chemistry and Preparation of Chemical Constituents of Tobacco Smoke.

In reviewing this proposal, I have divided the approaches suggested by Dr. Mandl into a number of headings. I will attempt to comment on aspects of each heading. The headings selected are:

a) The use of Tissue Culture to Study Protein Production.

b) The Effect of the Environment on Cells -- particularly as affected by the addition of chemicals -- as in smoke.

c) Aspects of Protein Chemistry -- particularly protein composition and structure.

It seems to me that the potential results that may be obtained from this project could be quite significant. While the course of development of the project will obviously rest heavily on the prime investigator, there is no question in my mind as to the basic capability of the investigators in this direction.

1003539760

Source: https://www.industrydocuments.ucsf.edu/docs/jjpl0000

When the project, if undertaken is finished, it should be possible to determine the effectiveness of Tissue Culture as a tool in this area of research. The effect of cross linking of natural structural polymers such as elastin and collagen as regards function and aging reaches back historically in the field of biochemistry. The effect of the current environment that man is exposed to is certainly pertinent. This could develop into a very interesting series, particularly if the wider aspects along with the necessary documented experimental details are kept in perspective.

Yours very sincerely,

Richard Holmes

Richard Holmes, Ph.D.
Associate Chief of Biochemistry

RH:ejm

Enclosure

10035397761

Source: https://www.industrydocuments.ucsf.edu/docs/jjpl0000

(a) Tissue Culture and Protein Production.

Cell cultures are conveniently divided into two categories (A) Primary cells isolated from fresh tissue and (B) Established cells which may be cultured indefinitely. In order to grow primary human cells successfully in Medium 199 or Ham's F12 serum must be added. The use of both these media has been suggested by Dr. Mandl along with added fetal calf serum or human placental serum. It should be noted that both types of sera are abnormal compared to the environment in which fibroblasts and epithelial cells are found in the lung. The proteins, hormones, particularly steroid hormones and a variety of small molecular substances are different, many unrecognized qualitatively and quantitatively. All these substances exert a profund effect on cellular metabolism. For this reason one might suggest that any sera used in tissue culture be monitored for proteins by acrylamide gel electrophoresis and by isoelectric focusing. Lipids can be monitored by thin layer or gas chromatography. That cell cultures take up proteins from the environment has been documented by Hamburger et al (1) and Ryser (2). Cells apparently adsorb and remove proteins from the medium particularly albumin. Dr. Mandl suggests oval albumin for stabilizing and dispersing chemicals found in smoke. The use of any albumin for this purpose requires some consideration of its chemistry, a brief reference to King (3) and Foster(4) reports on albumin will indicate the factors involved.

Much work in the field of tissue culture has been directed towards maximum stimulation of growth and the elimination of inhibitory or toxic substances. This point of view probably accounts for the extensive use of fetal calf serum, since it lacks many globulins and also the large lipoprotein fraction found in the adult. A case in point that the composition of the serum directly effects the metabolism of human cells is the recognition of so-called Rily bodies (5) in cells grown in fetal calf serum but not when cells are grown in human serum. These bodies are associated with certain glycoprotein disorders. Maximum rates of growth while convenient may be abnormal and lead to unbalanced metabolism. One might suggest that consideration be given to fully chemically defined medium such as described by Bakken et al (6) and Holmes et al (7). Established human cells may be grown as monolayers without difficulty and primaries may be grown out to confluency. That trypsin, used in the transfer of cell cultures, alters the nature of the cell membrane is known and recently documented by Burger (8). During this period before the cells have re-established their outer coat they may be affected by ingredients in smoke. The course of elastin or collagen production during growth from the time of inoculation to the time of confluency may be different. Dr. Mandl has indicated the role of ascorbic acid which raises the whole question of oxidation and reduction and the production of free radicals as related to the cross-linking of proteins. The role of lipoproteins, for example isolation and characterization of Lung surfactant by Steim (9) may have a significant effect on transport and uptake of chemicals.

10035397 62

Source: https://www.industrydocuments.ucsf.edu/docs/jjpl0000

(b) The Effect of Environment on Cells.

Many aspects of the environment as it affects cells could be discussed. Perhaps a pertinent aspect is the manner in which tobacco smoke ingredients are incorporated in the medium and some of the components in culture medium which may be affected. Many chemicals do not always enter cells readily and can be influenced by a second substance such as a carrier (albumin), a hormone (insulin) or one that affects ionic relationships (polylysine or polyglutamic), surfactants can be involved (phospholipids). In this regard the outer cell barrier (cell membrane) can be involved (see previous section). Alterations that are induced may result in changes from the limited growth potential of primaries to the unlimited growth of established cells. Under these circumstances it is not unreasonable to speculate that protein structure may be significantly altered.

Ascorbic acid, already commented upon in relation to oxidation-reduction, raises consideration of other reducing materials often used in culture media. The list of substances includes DPN, TPN, glutathione, cysteine (as opposed to cystine), lipoic (thioctic acid), alpha tocopherol and others. These materials, for example DPN (oxidized and reduced) are probably involved in the deamination and aldehyde condensation of lysine involving cross-linking of proteins, which Dr. Mandl proposes to examine. Glutathione has recently been shown by Meister (10) to be involved in transport, a cyclic system has been proposed. Thioctic acid introduced in culture media by Holmes was confirmed as a requirement for growth of human cells by Chang (11).

Tween 80 has been used in the past when a protein-free system was required, for dispersing hydrophobic substances. Most surfactants are highly toxic in tissue culture and certainly the chemical structure of Tween 80, polyethylene backbone, is not natural. As an alternate, cholic acid, as opposed to desoxycholic, has been proposed and used successfully for preparing lipid dispersions in tissue culture (7).

1003539763

Source: https://www.industrydocuments.ucsf.edu/docs/jjpl0000

(c) Protein Structure

This aspect is the highlight of Dr. Mandl's approach. It will be very interesting to discover if desmosine and isodesmosine linkages are affected by the environment since cystine in elastin appears to be constant at one half residue. Specifically, one may look for disturbances in the metabolism of lysine. While the insolubility of elastin limits electrophoretic studies this may not apply to enzymatically prepared fragments and indeed Dr. Mandl suggests looking at such fragments. Some criterion of purity of the fractions analysed will have to be developed in order to arrive at an understanding of the microheterogeneity of both elastin and collagen. Oxidation-reduction relationships in the cellular environment may affect condensation of lysine to desmosine and isodesmosine and to the formation of lysine nor leucine and merodesmosine. In this regard Paz et al's (12) report on the presence in elastin of possible cyclic precursors of desmosine and isodesmosine may be significant. Within a single culture the point at which cells are harvested early log phase or at confluency in the case of primaries could be relevent. With primaries which exhibit a limited life span of 60 to 65 cultures, the nature of protein in actively dividing cultures (6-8 subcultures) may be different to that found in older cultures (50-60 subcultures) (see Hayflick). Some relative changes in enzyme coding resulting in different Km values appears to occur as cells age according to Holliday and Tarrant (13). The same may also be true of elastin and collagen in cells as they age normally and different in cells under the influence of some of the ingredients of smoke. It would seem likely that the age of the tissue donor may affect the results, again as suggested by Hayflick. Other linkages in protein should not be ignored, for exmple di- and tri-tyrosine as indicated by LaBell et al (14).

In summary, it is conceivable that the expansion of knowledge of structure of both elastin and collagen could occupy the full time of the investigators. A balance will have to be struck between examining the effects caused by the environment and the structural studies.

(NOTE - While this report was in preparation, two current pertinent articles appeared which are worth mentioning. The first by Tanzer on "Cross linking of Collagen" in SCIENCE, May 11, 1973. The second by Blumenkrantz, et al on "Effect of Amino Acids on Collagen Biosynthesis", IN VITRO, Vol. 8, No. 5, March-April 1973).

1003539764

Source: https://www.industrydocuments.ucsf.edu/docs/jjpl0000

(1) Hamburger, R. N., D. A. Pious and S. F. Mills, 1963, Immunology 6: 439.

(2) Ryser, H. J. P. 1968, Science 159: 390

(3) Sogami, M., H. A. Petersen and J. F. Foster 1969, Biochemistry 8: 49.

also

Sogami, M. and J. F. Foster 1962, J.Biol. Chem. 237, 2514.

(4) King, T. P. and Spencer, E. M. 1970, J. Biol. Chem. 245, 6134.

(5) Danes and Bearn 1966, J. Exp. Med. 123, 1.

(6) Bakken, P. C., Evans, V. J., Earle, W. R. and Stevenson, R. E. 1961, Am. J. Hyg. 76, 96.

(7) Holmes, R. 1967, J. Cell Biol. 32, 297.

(8) Burger, M. M. 1973, Fed. Proc. 32, 91

(9) Stein, J. M. 1969, Biochem. & Biophys. Res. Comm. 34, 434

(10) Meister, A. 1973, Science 180, 33.

(11) Chang, R. S. and Humes, H. 1926, J. Exp. Med. 115, 937.

(12) Paz, M. A., P. M. Gallop, O. Olga Blumenfeld, E. Henson and S. Seifter 1971, Biochem. & Biophys. Res. Comm. 43, 289.

(13) Holliday, R. and G. M. Tarrant 1972, Nature 238, 26.

(14) LaBella, F., F. Heeley, S. Vivian and D. Thornhill 1967, Biochem. Biophys. Res. Commun. 26, 748.

(15) Tanzer, M. L. 1973, Science 180, 561.

10035397765

Source: https://www.industrydocuments.ucsf.edu/docs/jjpl0000